transducing the smooth muscle cells with a gene which encodes the protein product, operably linked to a promoter;

immobilizing on a tubular elongate porous vascular graft device the transduced smooth muscle cells within the pores and interior surface of the graft; and

coating the interior of the graft device having immobilized thereon the transduced smooth muscle cells with the endothelial cells.

C6

21. (Twice Amended) The method of claim 20, further comprising the step of cultivating the vascular smooth muscle cells obtained from a mammalian subject in a medium containing autologous serum prior to immobilizing the cells on the vascular graft.

C7

22. (Amended) The method of claim 21, further comprising the step of cultivating the vascular endothelial cells obtained from a mammalian subject in a medium containing autologous serum prior to coating the vascular graft.

REMARKS

With entry of this amendment, claims 1-22 are pending in the application. By this amendment, claims 1, 10, 11, 13-16, and 19-22 have been amended for clarity and in accordance with the Office's suggestions. All of the amendments presented herein are fully supported by the specification and no new matter has been added to the application. Entry of this amendment is respectfully requested.

Concerning the amendments to claims 1 and 10, above, the changes clarify certain alternate configurations of Applicants' device. Claim 1 is presently directed to a "device for implanting autologous vascular smooth muscle cells transduced with a gene of interest". The basic construction of this device is "a tubular elongate member having a wall, which wall has an interior surface, an exterior surface, and pores therein; and autologous smooth muscle cells transduced with the gene of interest immobilized within the pores and upon the interior surface of the wall to form a tubular smooth muscle cell complex." The claims have been further clarified in this context to recite that "the smooth muscle cells remain stably immobilized on the graft surface and express a product of said gene", as clearly disclosed in the specification.

The language in original claim 1 pertaining to incorporation of endothelial cells in the vascular graft has now been incorporated in dependent claim 10, which recites the device of claim 1 wherein, "the device, prior to implantation in a subject, further comprises autologous vascular endothelial cells adherent to an interior surface of the tubular smooth muscle cell complex." This change clarifies that the device of claim 1 does not require an *ex vivo*-supplied layer of autologous vascular endothelial cells to function within the invention. Recruitment of endothelial cells can be alternately achieved, and in fact naturally transpires, to provide this feature of the device, post-implantation. Moreover, these cells are not essential to define or enable the devices presently set forth in the claims. This change follows the Office's focus of review with regard to essential subject matter that distinguishes the invention over the prior art. At the same time, this change is reflexive to the Office's focus regarding enablement criteria pertaining to Applicants' "gene therapy" embodiments versus the present device claims. These changes parallel the subject matter of newly amended claims 11-22, which have also been amended in response to the Office's concerns regarding enablement of "gene therapy" methods. Briefly, claims 11-22 have been amended herein to recite "[a] method for preparing a vascular prosthesis", which amendments are intended to hold difficult prosecution issues regarding enablement of gene therapy claims in abeyance for prosecution in a future, related application.

Patentability Under 35 U.S.C. § 112, First Paragraph

The specification stands objected to and claims 11-22 rejected for alleged lack of enablement under 35 U.S.C. § 112, first paragraph. The primary grounds for this rejection asserted by the Office is that the art of gene therapy is highly unpredictable and that there is a lack of correlatable working examples in the specification to support the scope of the claims as they relate specifically to gene therapeutic methods.

Applicants respectfully traverse the stated grounds for rejection based on the evidence and remarks set forth in the record. Briefly, Applicants submit that methods broadly characterized by the Office as "gene therapy" methods, that employ the novel vascular prosthetic devices of the invention, are fully enabled in view of the novel techniques and

advantages set forth in the record. In this context, Applicants submit that the nature of the invention represented by the original claims departs in substantial ways from the general field of gene therapy on which the Office's criticisms are based. As previously noted in the record, Applicants' original claims were distinctly directed to a proven method for delivering useful genes and their products to mammalian subjects using vessel grafts seeded with transduced smooth muscle cells. The specification provides detailed teachings that enable smooth muscle cell transduction and graft seeding, and long term expression of representative genes, as demonstrated both *in vitro*, and *in vivo* using accepted animal models. Further evidence in the form of additional studies that follow Applicant's teachings in the specification confirm the efficacy of the claimed methods. As discussed further in the record, these unique methods and materials overcome many of the purported obstacles and uncertainties identified by the Office "highly unpredictable" factors characterizing the general field of gene therapy.

At the same time, Applicants respectfully submit that the policies and procedures of the Office with regard to examination of gene therapy applications remain poorly resolved. A survey of PTO guidelines and issued gene therapy patents fails to provide any clear standard for enabling claim support in this technical field. This attendant uncertainty presently facing Applicants warrants deferral of this aspect of the invention for prosecution in a future, related application. On this basis, the allegedly objectionable gene therapy subject matter has been withdrawn from consideration in the instant application, without prejudice and with full reservation to carry this subject matter forward in a subsequent, related case.

Thus, for the purpose of clarity and in the interest of advancing the present case to issuance, Applicants have amended the claims herein to redirect the Office's attention to two distinct aspects of the invention. The first aspect relates to "[a] device for implanting autologous vascular smooth muscle cells transduced with a gene of interest into a mammalian subject" (claim 1). The Office has already considered this subject matter and deemed it to be allowable under 35 U.S.C. § 112, first paragraph. In particular, in Office Action Paper No. 15, the Examiner stated that: "The rejection based upon the first paragraph of 35 U.S.C. § 112 is hereby considered withdrawn, as it relates to claims 1-10, in light of the response filed 2-11-97 (paper #14)".

The second aspect of the invention now presented for consideration relates to the foregoing device claims as "[a] method for preparing a vascular prosthesis seeded *ex vivo* with vascular smooth muscle cells transduced to express a gene of interest." It is respectfully submitted that these amendments obviate the issues raised under 35 U.S.C. § 112, first paragraph pertaining to enablement of gene therapy aspects of the invention. In this regard, the Office has expressly acknowledged that the specification sets forth detailed examples documenting smooth muscle cell transduction, successful graft seeding, and stable gene expression by Applicant's seeded vessel grafts. Thus, the specification clearly satisfies the principal requirement for enablement of the amended claims, which is that there must be a "reasonable correlation" between the disclosure and the scope of the claims. (See, e.g., In re Vaeck, 20 USPQ2d 1438 (Fed. Cir. 1991); Ex parte Jackson, 217 USPQ 804, 807 (Bd.Pat.App.Int. 1982); In re Fisher, 166 USPQ 18, 24 (CCPA 1970); MPEP § 706.03(n)).

To further clarify these issues, Applicants refer to their response filed 2-11-97 (paper #14), and to Example G "Gene Therapy" presented in the Training Materials for Examining Patent Applications With Respect to 35 U.S.C. Section 112, First Paragraph-Enablement Chemical/Biotechnical Applications (hereinafter "Enablement Guidelines"). In this example, a hypothetical disclosure describes viral vectors for delivering genes of interest into mammalian cells. Sample vectors incorporating various genes of interest are provided and shown to infect cells and cause them to produce corresponding proteins of interest. According to the Guidelines under this type of scenario, claims to the viral vector (and, presumably to methods for producing the vector) which do not recite an *in vivo* use or treatment utility are considered allowable, on the basis that, "because it does not recite any environment of use, only one enabled use covering the scope of the claim is needed to enable the claim." For analogous reasons, Applicants' claims 11-22 directed to methods for preparing a vascular prosthesis should clearly be allowable.

In view of the foregoing, Applicants respectfully submit that all of the pending claims, as amended herein, completely fulfill the requirements for patentability under 35 U.S.C. § 112, first paragraph.

Patentability Under 35 U.S.C. § 103

Claims 1-10 stand rejected under 35 U.S.C. § 103 as allegedly unpatentable over Zalewski et al. taken with Nabel et al and Anderson et al.

Applicants respectfully traverse the stated grounds for rejection for reasons of record, and further based on the supplemental evidence and remarks submitted herewith in the Declaration of Dr. William Osborne (Osborne Declaration). The evidence presented in the Osborne Declaration, and the opinion testimony presented by Dr. Osborne therein, are summarized below for formal consideration and entry in the record.

As an initial point for consideration, Applicants respectfully request that the Office consider Dr. Osborne's Declaration as being generally representative of the level of scientific knowledge, skill, and reasoning held by ordinarily skilled artisan's in the field of the invention. Dr. Osborne's *curriculum vitae* is appended to his Declaration. Consideration of this material (see also, Osborne Declaration at ¶¶ 1-6), along with the detailed remarks provided in Dr. Osborne's Declaration, are believed to validate this status.

As noted in Dr. Osborne's Declaration (¶ 8), the Office has cited the Zalewski et al. reference as teaching certain aspects of Applicants' invention, including:

> transformation of smooth muscle cells on page 8 using various gene transfer methodologies. Further, page 9 of the reference discloses the use of implant devices to hold and contain said vascular smooth muscle cells. (Office Action Paper No. 11, at p. 10).

Dr. Osborne's Declaration (¶ 9) provides a considerably different interpretation of this disclosure. In particular, it is noted that the Zalewski et al. reference focuses solely on *in situ* transduction methods for vascular gene therapy. There is no disclosure "implant devices to hold and contain said vascular smooth muscle cells." The only devices and methods taught by Zalewski et al. are "injection and transcatheter delivery devices to deliver a solution" or a "perfusate" under pressure, containing genes and vectors to transduce smooth muscle cells

in arteries *in situ* (see, e.g. page 8, lines 21-23; page 9, lines 14-35, page 10, lines 4-8). The proposed use of these devices is transitory, lasting 1-2 minutes. As emphasized by Dr. Osborne, this limited window of time for inserting the perfusion catheter is imposed by the risk of myocardial infarction (see, e.g. page 10, lines 4-8).

There Zalewski reference is therefore not considered to disclose or suggest devices or methods "to hold and contain" transduced smooth muscle cells (SMCs) as argued by the Office, particularly in the manner achieved by the claimed prosthetic devices. These devices are seeded with SMCs *ex vivo*, after the cells are transduced *ex vivo*. The traduced SMCs are not administered or "instilled" directly, but are implanted as a seed cell layer component of a long term graft.

As further detailed in Dr. Osborne's Declaration (¶ 9) the Zalewski et al. reference is considered to actually teach away from long-term graft devices as presently claimed. In particular, the reference discloses perfusion catheters which are taught as delivery vehicles for solutions and perfusates, not cells. The catheters function only on a very transient delivery basis. In contrast, the presently claimed devices and methods deliver transduced SMCs integrated in a prosthetic implant that physically replaces or bypasses existing vessels.

Because the teachings of Zalewski et al. focus the artisan's attention on *in situ* transduction methods for vascular gene therapy, the reference:

> strongly influences the "direction" and extent of "motivation" that a skilled artisan would have gleaned from the reference to embark on a course leading toward, or away from, the presently claimed invention. From my analysis of the facts, I conclude that the Zalewski et al. reference teaches directly away from long-term delivery engrafting devices and methods as presently claimed. (Osborne Declaration at ¶ 10, emphasis in original).

As further explained in Dr. Osborne's Declaration, the focus of Zalewski et al. on *in situ* transduction devices and methods are supplemented by numerous additional publications in the literature which similarly depart in the fundamental course of their teachings from the devices and methods of the present invention. "These reports include a considerable assemblage of articles that expressly favor *in situ* over *ex vivo* transduction

methods for implementing vascular gene therapy." (Osborne Declaration at ¶11). Among these reports noted by Dr. Osborne is the article by Nabel et al., Science 249:1285-1288, 1990 (of record), which is considered to refute the proposed utility of implanting *in vitro* transduced endothelial cells mentioned as an alternative gene therapy method in the Nabel et al. patent (U.S. Patent No. 5,328,470) cited by the Examiner. In this article, Nabel and coworkers expressly criticize their earlier vascular gene therapy studies that focused on *ex vivo* transduction and reimplantation of genetically modified endothelial cells. In relevant part noted by Dr. Osborne, the authors state that:

> Because these studies required that syngeneic cell lines be established before genetic modification, adaptation to the treatment of human disease remained cumbersome. We now report that a recombinant gene can be efficiently expressed at a specific site in vivo by direct introduction of genetic material at the time of catheterization. (page 1285, middle and right columns, emphasis added).

In paragraph 12 of his Declaration, Dr. Osborne points to similar teachings in the Nabel et al. patent cited by the Office, wherein "the inventors point to a distinct advantage that can be achieved through 'introduction of recombinant genes directly' *in vivo* (as opposed to *ex vivo* transduction)." Reference is further provided to the Nabel et al. patent disclosure involving direct gene transfer (for cancer treatment) in comparison to "traditional", *in vitro* gene transfer techniques. In this context, Dr. Osborne cites (at ¶ 12) the following passage from the reference:

> The prior art approaches (referring specifically to "modification of tumor cells in vitro followed by transfer of the modified cells") are disadvantageous because they subject the cells to selection in different growth conditions from those which act in vivo, and because they also require that cell lines be established for each malignancy, thereby rendering adaptability to human disease considerably more difficult. (column 12, lines 34-45, underscoring added to relevant text).

Evaluating this disclosure from the perspective of the ordinarily skilled artisan, Dr. Osborne states (at ¶ 13) that:

> These teachings lead directly away from *ex vivo* transduction of SMCs and *ex vivo* seeding of transduced SMCs onto a prosthetic vascular implant, as presently claimed. There is an express teaching against *ex vivo* transduction techniques. At the same time, there is an implicit teaching against the use of any kind of implantation device or method to introduce transduced cells for vascular gene therapy. This skepticism forecast by Nabel et al. with regard to "traditional" *in vitro* gene therapy methods reflects a widely adopted perspective in the art at the time of the invention. This perspective, expressly favoring direct, *in vivo* transduction methods for vascular gene therapy, proved to be strongly influential in the art, as evinced by more recent reports.

In further support of this position, Dr. Osborne cites (at ¶ 13) a later published article by Kuo et al., Am. J. Roentgenology 171:553-558, 1998, which is considered to "teach directly away from *ex vivo* transduction and implantation devices for vascular gene therapy, as follows":

> The most advanced tissue engineering strategies currently available are cell-based in vitro studies or simplistic ex vivo strategies. These strategies are by their very nature inefficient, somewhat awkward, and thus of limited clinical usefulness . . ..
>
> By transfecting the desired vein segment with the Adv/RSV-tPA construct in situ, we were able to confer the desired thrombolytic characteristics to the graft in vivo, avoiding the need for complex ex vivo or in vitro treatments. (page 556, emphasis added).

By considering the foregoing evidence, Dr. Osborne presents a conclusion in paragraph 14 of his Declaration that:

> [T]here does not appear to be a scientifically well-founded "suggestion" or "motivation" provided by the cited references, taken as a whole, that would have led the artisan to independently create the presently claimed devices and methods. Like Zalewski et al., Nabel et al. clearly teach that *in situ* transduction methods are favored, leading directly away from the combination recited in the claims. At the same time, the exact combination of teachings presently relied by the Examiner remains unclear from the record. As discussed above, Zalewski et al. appears to been originally cited in error, for allegedly teaching "the use of implant devices to hold and contain said

> vascular smooth muscle cells." (Office Action Paper No. 11, at p. 10).

The next passages of the Osborne Declaration focus on the Office's interpretation of the Nabel reference, addressing particularly the following statement in Office Action Paper No. 26:

> The fact that the Zalewski et al. reference does not specifically disclose an implantable prosthetic device lined with SMC (smooth muscle cells) does not take away from the fact that the Nabel reference does. (Paper No. 26, at page 4, underscore added).

Considering this statement, Dr. Osborne concludes (in ¶ 15) that the Examiner's interpretation of Nabel et al. appears in error, and that the teachings of Nabel et al. are expressly limited to the use of "catheter means" "for the instillation of vectors or cells" (citing, e.g., columns 7 and 8 of Nabel). It is further concluded that, even when cells are transduced *ex vivo*, Nabel does not teach the use of an "implantable prosthetic device" that is "lined with" transduced cells. On the contrary, Dr. Osborne points to column 7 of Nabel et al., where the it is stated that: "After instillation of the infected endothelial cells, the balloon catheter is removed . . .." On this basis, Dr. Osborne concludes that "reference must be considered to teach directly away from 'implantable device' lined with SMC" or any other kind of cells". This statement is followed by yet another reference teaching from a subsequent Science article by Nabel et al. (supra, at page 1286), which Dr. Osborne notes to be critical of the authors' prior method employing a catheter to instill *ex vivo* transduced endothelial cells. The relevant teaching in the later Nabel article is cited, and reproduced here, as follows:

> Although this method was effective, it required that cells syngeneic to the recipient animal be prepared and transduced, which took several weeks to prepare. Direct retroviral infection and liposome transfection allow the introduction of recombinant genes into any site accessible to a catheter without advanced preparation . . . this approach minimizes potential complications . . .. (emphasis added).

Based on this evidence, Dr. Osborne concludes that the Nabel et al. and Zalewski et al. references teach away from the use of *ex vivo* vascular cell transduction and,

separately or in combination, the use of a prosthetic implant lined with transduced cells (see, ¶ 16).

The next focus of discussion by Dr. Osborne is the Anderson et al. publication (WO 90/224,525). The Examiner correctly notes that this reference is limited in its description to using "genetically engineered endothelial cells and the use thereof for expressing a therapeutic agent" (Anderson et al., at p. 1, Office Action Paper No. 26 at page 4, underscore added). As a preliminary point in discussing this reference, Dr. Osborne notes that the disclosure purports to offer a large array of useful, alternative "solid supports" for transduced endothelial cells. These alternative supports include vascular shunts and by-passes, pads, strips, gels and other compatible implants (see, e.g., page 5). As stated in the accompanying Declaration (at ¶ 17), "this broad spectrum of allegedly useful "implant" devices detracts from any proposed teaching that relates to the presently claimed vascular grafts." Further addressing the deficiencies of this reference, Dr. Osborne notes, that the "Examples" provided by Anderson et al. relating to the present invention are limited to a brief description and evaluation of transduced endothelial cells in culture.

> The only Example that pertains directly to a seeded "graft" is a limited, *in vitro* Example wherein the seeded graft was maintained and assayed strictly in a tube of culture medium with no evidence of *in vivo* viability or transgene expression (see, Example 1, e.g., at page 12). This limited teaching does not, in my interpretation, support the Examiner's contention that Anderson et al.: 'discloses to the skilled artisan a vascular graft coated with genetically modified autologous endothelial cells, and further discloses the use of this invention to deliver erythropoietin, Factor IX, G-CSF and GM-CSF proteins, among others. (Paper No. 26, at page 4).' (Osborne Declaration at ¶ 18)

Discussing the reference in further detail, Dr. Osborne states at paragraph 19 of his Declaration that "I do not believe that the Anderson et al. reference would have been interpreted by the skilled artisan as providing an effective vascular graft for gene therapy in the manner alleged by the Examiner. I am also puzzled as to how the Examiner's interpretation of Anderson et al. is reconciled with the Examiner's own rejections and technical concerns raised in the Office Action under 35 U.S.C. § 112, first paragraph." Clarification of these issues is

requested to assist Applicants in presenting *in vivo* gene therapy aspects of the present invention presently withdrawn from consideration by this Amendment, without prejudice.

A summary analysis of the Anderson et al. reference is provided in by Dr. Osborne in his Declaration, as follows:

> The limited teachings of Anderson et al. do not provide sufficient scientific motivation or guidance to overcome the negative teachings of Zalewski et al., Nabel et al., and others, noted above, that teach away from the use of *ex vivo* cellular transduction and instillation in any form, as well as the more unpredictable task of *ex vivo* graft seeding and implantation, in favor of direct, *in situ* transduction methods. More importantly, Anderson et al. provides nothing that would lead the artisan to substitute SMCs for the designated preferred, endothelial cell targets for transduction, seeding and/or implantation, allegedly described in the reference. Even if one skilled in the art accepted the teachings of Anderson et al. (considering the limited working examples noted above), to evince successful transduction, seeding and implantation of endothelial cell-coated vascular grafts, this acceptance would not translate to a "reasonable expectation of success" for extending these teachings to achieve the presently claimed vascular grafts incorporating transduced SMCs. This is particularly if one considers the distinct challenges and uncertainties involved in SMC and endothelial cell culture, transduction, seeding, and prolonged viability and transgene expression *in vivo*. (Osborne Declaration at ¶ 20, emphasis supplied).

In the following paragraphs, Dr. Osborne concludes that, even if Anderson et al. and other publications are accepted as teaching a successful vascular graft seeded with transduced endothelial cells suitable for long term implantation and expression of a foreign gene, "there is no sufficient teaching or suggestion in the art that would have lead the ordinarily skilled practitioner, at the time of the invention, to substitute transduced SMCs for endothelial cells in a vascular graft implant, as the Examiner proposes." (¶ 21, underscore added).

On the contrary, Dr. Osborne points to "numerous, independent grounds to support my conclusion that the art teaches directly against making the proposed substitution of cell types in a vascular graft. As an initial point for consideration, Dr. Osborne notes that:

> [I]f the device of Anderson et al. is actually useful in the manner advocated by the Examiner, *it would be counterintuitive to substitute SMCs for endothelial cells to arrive at the presently claimed invention*. It is a fundamental principal of scientific reasoning that one should not alter a proven device or system demonstrated to work for an intended purpose, absent some compelling, practical motivation to do so. It is another basic scientific tenet not to increase the complexity of a proven device or system, without some well-reasoned expectation of substantially improved results. Thus, if Anderson et al. in fact teaches a useful vascular graft seeded with transduced endothelial cells for long term implantation and expression, *one would be directly countermotivated to substitute SMCs for endothelial cells in such a useful implant, contrary to the Examiner's suggestion*. (Osborne Declaration at ¶ 22, italics added).

To further support these conclusion, Dr. Osborne notes that numerous additional references in the record "follow the same direction as allegedly taught by Anderson et al., by reporting transfection of endothelial cells, the use of endothelial cells to line vascular grafts, viability of endothelial cell-lined grafts in vivo, and/or development of vascular grafts seeded with transduced endothelial cells." (id.) In particular, the Examiner's attention is directed to Dichek et al., Circulation 80:1347-1353, 1989, and Flugelman, Thromb. Haemost. 74:406-410, 1995 (copy enclosed for consideration and entry in the record). Dichek et al. reports successful coating of retroviral-transduced endothelial cells onto stainless steel stents. The reference does not provide *in vivo* data, but instead cites Wilson et al. and Nabel et al. as having "reported encouraging data on the ability of implanted transduced endothelial cells to survive and proliferate in vivo." (page 1352, left column). Flugelman is similarly cited for teaching "the use of genetically engineered endothelial cells to improve the surface of a metallic endovascular prosthesis known as a stent." (page 406, right column).

In light of these and other reports, Dr. Osborne concludes in ¶ 23 of his Declaration that:

> [T]here does not appear to be any direct, practical suggestion or compelling motivation in the art of record to make the proposed substitution of SMCs for endothelial cells in a prosthetic vascular graft for in vivo gene therapy, as proposed by the Examiner. On the contrary, sound scientific reasoning, and a consensus of teachings noted above, would appear to direct otherwise.

In support of this conclusion, Dr. Osborne notes that the record discloses, "or is at least advocated by the Examiner to disclose", that: (1) endothelial cells are an "excellent target" for gene therapy, in part because they line the vascular lumen and thus provide the advantage of direct exposure (of the transduced cells and their secreted products) to the circulation; (2) endothelial cells are reportedly shown by Anderson et al. and others to be readily transduced and seeded onto vascular graft surfaces; and (3) seeded endothelial cells on vascular grafts have been demonstrated to exhibit long-term survival *in vivo*. Specific references and citations are next offered by Dr. Osborne to confirm these differences relating to the proposed use of SMCs as substitutes for endothelial cells in gene therapy and, more specifically, in the context of transduced cell-seeded, vascular grafts for *in vivo* transgene expression. For completeness of the record, these passages from Dr. Osborne's Declaration (¶¶ 24-28) are reproduced here.

With regard to the prior art teaching a clear preference for endothelial cell targets in this context, Welling et al., Hum. Gene Ther. 7:1795-1802, 1996 (copy enclosed) states as follows:

> Endothelial cells are considered an excellent target for gene transfer because the represent a durable tissue located strategically at the blood tissue interface. A number of investigators have successfully transduced the endothelium of large muscular arteries (Nable and Nabel, 1994; Messina et al., 1995) (p. 1796, left column).

25. Nabel et al., U.S. Patent No. 5,328,470 (of record), teach that direct, *in situ* transduction of endothelial cells provides the following advantages:

> In this way, the recombinant genes may be secreted directly into the circulation which perfuse the involved tissue or may be synthesized directly within the organ. (column 5, lines 19-22).

26. Similarly, Zweibel et al., Science 243:220-222, 1989 (copy enclosed), teach that:

> The endothelium, because of its contiguity with the bloodstream, is a particularly attractive target for the delivery of functional genes in vivo. The use of endothelium for gene transfer would permit secretion of a recombinant protein from genetically engineered endothelial cells directly into the blood. (page 22, right column, underscore added).

27. Reiterating and affirming these teachings, Zwiebel and other coworkers stated in Biochem. Biophys. Res. Comm. 170:209-213, 1990 (copy enclosed) that:

> Endothelial cells are attractive targets for gene transfer because of their immediate contact with the bloodstream, and, therefore, they might serve as attractive targets for therapeutic drug delivery. . . .. The fact that a recombinant gene can be readily inserted and efficiently expressed into human endothelial cells suggests that these cells may be able to serve a role in human gene therapy. (page 209, Abstract, emphasis supplied).

28. Further validating these teachings, Wilson et al., Science 244:1344-1346, 1989 (of record), state that:

> Because of their proximity to the blood stream endothelial cells are an **obvious** candidate for delivering therapeutic proteins systemically. (page 1346, left column, emphasis added).

Considering this evidence Dr. Osborne emphasizes a "direct contrast" between these teachings and the instantly claimed invention, stating in particular that:

> SMCs were not viewed in the art at the time of the invention to be an "excellent target" for gene therapy. Moreover, SMCs are not naturally in direct contact with the circulating blood, but are instead covered by endothelial cells that would at least impair the exposure of transduced SMCs to the circulation to yield a therapeutic influence following transgene expression. Finally, SMCs had not been shown at the time of the invention to be readily transduced and seeded onto vascular graft surfaces, nor had their by long-term survival as a transduced, seeded cell layer on vascular grafts been established. (Osborne Declaration at ¶ 29, underscores added).

Additional evidence is provided in Dr. Osborne's Declaration (e.g., at ¶¶ 30-36) to show that SMCs and endothelial cells would not have been viewed to be "interchangeable" in a vascular graft. In other words, SMCs would not have been considered "as an equivalent substitute for endothelial cells." It is thereby proposed that:

> [T]o include SMCs as a component of a vascular graft as advocated by the Examiner, rather than simply substituting one cell type for the other to make the proposed combination, the artisan would need to assemble a combination of multiple cell types in the graft, rendering the proposed combination far more complex to engineer and much less predictable. This follows the teachings noted above that endothelial cells are an "excellent target" for gene therapy. In addition, there was a long-standing consensus in the art at the time of the invention that endothelial cells are an important or essential component of vascular implants, to prevent thrombosis and other adverse effects and otherwise better mimic natural blood vessel structure/function. (Osborne Declaration at ¶ 30, emphasis supplied).

In support of this position, Dr. Osborne cites Welch et al., Ann. Vasc. Surg., 6:473-484, 1992, which reviewed a broad spectrum of literature relating to endothelial vascular graft seeding, and disclosed that:

> Attempts to improve prosthetic graft performance have progressed broadly along two fronts: mechanical and biological. The latter adopts the concept that improved performance could be achieved if the luminal surface of the graft had biological characteristics of normal vessels, being lined with endothelium capable of resisting platelet aggregation. (page 473, left column, emphasis added). . . .. These original experiments have since generated a large volume of research to develop a technique to line prosthetic grafts with a confluent functional endothelial cell monolayer. (id., right column).

Also cited in paragraph 31 of Dr. Osborne's Declaration is a supporting reference by Vohra et al., Eur. J. Vasc. Surg. 5:93-103, 1991, teaching that:

> In order to overcome the thrombogenecity of synthetic vascular prostheses, attempts have been made to line these grafts with living endothelium.[1-3] Animal studies have shown reduced platelet adhesion and improved patency in endothelial cell seeded grafts.[4-6] Dacron and Polytetrafluoroethylene have both

> been successfully seeded with endothelial cells resulting in confluent monolayers in vitro as well as in vivo.[2,3,6-9]

In conjunction with this evidence, Dr. Osborne points to a separate line of teachings in the art that disclose "one of the principal benefits of endothelial cell seeding of vascular grafts has been considered to be the prevention of smooth muscle cell proliferation in grafts to prevent graft occlusion." (Osborne Declaration at ¶ 32). This and related teachings in the art are deemed to "further direct the artisan away from incorporating seeded SMCs in vascular graft implants." This conclusion is explained partially on the grounds that:

> *[A] principal drawback in vascular graft surgery is* restenosis and other forms of neointimal hyperplasia *mediated by excessive proliferation of SMCs at sites of vascular graft implantation*." After the implantation of a vascular graft, aberrant recruitment and *growth of SMCs commonly narrows or occludes the vessel* lumen, leading to a loss of patency and/or graft failure. In light of these concerns, it is a widely proposed goal in the art to block SMC recruitment or growth at sites of surgical vascular injury, including vessel grafts. (id., italics added).

Support for these conclusions include references to Isner, U.S. Patent No. 5,830,879 (copy enclosed for consideration and entry in the record), cited for teaching a method for reendothelialization of the lining of an injured blood vessel using *in situ* transfection of DNA encoding vascular endothelial growth factor (VEGF). As noted by Dr. Osborne (id.), the stated purpose for this method is that it "inhibits smooth muscle cell proliferation and consequently reduces restenosis." (citing the Abstract, underscore added). Also referenced by Dr. Osborne is McCarthy, Lancet 347:752, 1996 (copy enclosed for consideration and entry in the record). This reference cites Isner's work involving in situ VEGF transduction of endothelial cells, specifying that the goal of the work is to both "stimulate endothelial proliferation" and "limit smooth-muscle-cell proliferation and other changes that cause restenosis." (emphasis supplied).

Yet additional teachings are detailed by Dr. Osborne which are concluded in his Declaration (e.g., at ¶¶ 33-36) to "teach away from using SMCs, or a combination of endothelial cells and SMCs, as a component for seeding a vascular prosthesis." Thus, Mann et al., Proc. Nat. Acad. Sci. USA 92:4502-4506, 1995 (copy enclosed for consideration and entry in the record) is cited for teaching a modified vascular bioprosthesis genetically engineered "specifically to block SMC growth." More specifically, Dr. Osborne directs to Office to page 4502 (Abstract) of the reference, where Mann and colleagues teach that:

> an intraoperative gene therapy approach using antisense oligodeoxynucleotide blockage of medial smooth muscle cell proliferation can prevent the accelerated atherosclerosis that is responsible for autologous vein graft failure. (underscore added).

These articles are considered by Dr. Osborne (¶ 34) to "specifically teach seeding of transduced endothelial cells in vascular grafts to inhibit SMC proliferation", and are thereby deemed "particularly relevant to the present analysis." As stated in the Declaration, "[t]hese articles can only be considered to teach directly away from seeding grafts with SMCs, alone or in combination of endothelial cells and SMCs."

Further reference in this context is made to Dichek et al., at page 1347 (Abstract) cited by Dr. Osborne (at ¶ 34) for teaching that:

> The use of intravascular stents may be limited by both local thrombosis and restenosis due to intimal proliferation. In an effort to provide solutions to these problems, we seeded stents with genetically engineered endothelial cells . . . (for) improvement of stent function through localized delivery of anticoagulant, thrombolytic, or antiproliferative molecules. (underscores added).

Similarly, Dr. Osborne (at ¶ 35) cites Wilson et al., WO 89/05345 (copy enclosed for consideration and entry in the record), for teaching that:

> There are many advantages to endothelial cells of the present invention. For example, they can be designed to improve the characteristics of endothelial cell-lined prosthetic implants by enhancing or improving the ability of endothelial cells to seed or

> bind to the inner surface of the implant; by modifying the endothelial cells used to line an implant so that they will grow; or by overcoming the problem, encountered with presently-available implants, of smooth muscle cell growth at the implant ends, which results in narrowing, and, ultimately, closing off of the ends. (page 4, lines 11-20, emphasis added) . . . (for example by) secretion of an inhibitor of smooth muscle proliferation to prevent luminal stenosis due to smooth muscle hypertrophy. (page 5, lines 5-7, underscore added).

Dr. Osborne's restated conclusion based on these references is that they "teach directly away from seeding grafts with SMCs, alone or in combination with endothelial cells." (Osborne Declaration at ¶ 36). It is further asserted that "[t]he Wilson et al. reference, because it teaches methods for 'improving the ability of endothelial cells to seed or bind to the inner surface of the implant', further teaches away from layering endothelial cells (i.e., in combination) over a graft initially seeded with SMCs."

In summary of this evidence, Dr. Osborne concludes (¶ 37) that:

> In light of all of the foregoing evidence and remarks, I conclude that the art of record, taken for what it teaches as a whole, fails to provide sufficient motivation and direction that would have led a person of ordinary skill in the art, at the time of the present invention, to make and practice the invention as claimed.

In view of the foregoing evidence and remarks, Applicants respectfully submit that the art of record fails to teach or suggest the presently claimed subject matter, and in fact teaches away from Applicants' novel devices and methods. The foregoing evidence and testimony of Dr. Osborne provide important indicia of non-obviousness, which must be considered in evaluating the patentability of the Applicant's invention. In this regard, the panel in In re Braat, 16 USPQ2d 1812 (Fed. Cir. 1990) emphasized that "[a] reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a path divergent from that taken by the applicant." As further explained in In re Gurley, 31 USPQ2d 1130, 1131 (Fed. Cir. 1994, emphasis supplied): "[I]n general, a reference will teach away if it suggests that the line of development flowing from the reference's disclosure is unlikely to be productive of the

result sought by the applicant." (underscore added). Further, a reference teaches away "if it leaves the impression that the product would not have the property sought by the applicant." (emphasis added). Each of the publications cited above and referred to by Dr. Osborne as "teaching away" from the presently claimed invention fulfills one or all of these tests articulated by the Federal Circuit. In this regard, the court has stated that, as a "useful general rule", "a reference that `teaches away' can not serve to create a prima facie case of obviousness." (id.) For these reasons, it is respectfully requested that the rejection of claims 1-10 under 35 U.S.C. § 103 be withdrawn.

As an alternative basis for overcoming the rejection, Applicants have submitted herewith in the form of Dr. Osborne's Declaration, detailed evidence to establish that the results achieved by the claimed invention represented "unexpected results", recognized by the Office as a sufficient basis to overcome a prima facie rejection under 35 U.S.C. § 103. These results are detailed in paragraphs 38-48 of Dr. Osborne's Declaration, which material is incorporated herein in its entirety. From the detailed scientific evidence provided in these passages of his Declaration, Dr. Osborne concludes (¶ 48) that:

> The foregoing evidence clearly demonstrates that the devices and methods of the invention, as described in the specification and as set forth in the pending claims, provide "unexpected results" over the prior art of record, viewed for what it teaches as a whole (as discussed above). In particular, even if it is considered that the art "suggests" to make the devices of the invention as claimed, there is no reasonable expectation of success that can be gleaned from these references to obtain the disclosed results, on an individual or collective basis.

In the instant case, even if a *prima facie* case of obviousness were established to support the rejection of claims 1-10, which Applicants do not concede, the burden shifts to the PTO to provide persuasive factual evidence to refute the evidence and conclusions provided in Dr. Osborne's Declaration demonstrating that the invention provides "unexpected results." As the Federal Circuit held in In re Soni, 34 USPQ2d 1684, 1688 (1995).

> [W]hen an applicant demonstrates *substantially* improved results, as Soni did here, and *states* that the results were

> *unexpected*, this should suffice to establish unexpected results *in the absence* of evidence to the contrary. (emphasis in original).

In Soni, the Federal Circuit panel rejected the Board's reasoning on the grounds that, even a cited reference taught a general relationship between polymer matrices and mechanical properties of composite systems, it failed to teach that specific, "higher molecular weight polymers form improved conductive polymer compositions.". (id., underscores added). Considering the present facts, the art of record similarly fails to forecast the results provided by the presently claimed invention, detailed in Dr. Osborne's Declaration. In this regard, it is "critical" for resolving the issue of obviousness that the Office must consider "the particular results achieved" by the new combination provided in the invention. Interconnect Planning Corp. v. Feil, 227 USPQ 543 (Fed. Cir. 1985).

In the instant case, the Office has proffered no evidence to refute the stated results detailed in Dr. Osborne's Declaration, whereby the record is clear that the invention provides "unexpected results" over the art of record. Absent a showing by the Office that contravenes this position, any *prima facie* case of obviousness against Applicants' claims must be deemed to have been satisfactorily addressed and overcome.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: March 7, 2001

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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

1. (Twice Amended) A device for implanting autologous vascular smooth muscle cells transduced with a gene of interest into a mammalian subject, comprising:

a tubular elongate member having a wall, which wall has an interior surface, an exterior surface, and pores therein; and

[the] autologous smooth muscle cells transduced with the gene of interest immobilized within the pores and upon the interior surface of the wall to form a tubular smooth muscle cell complex [having an interior surface; and

autologous vascular endothelial cells adherent to the interior surface of the tubular smooth muscle cell complex] whereby the smooth muscle cells remain stably immobilized on the graft surface and express a product of said gene.

10. (Amended) A device as in claim [9] 1, wherein the [polymer is collagen or fibronectin] device, prior to implantation in a subject, further comprises autologous vascular endothelial cells adherent to an interior surface of the tubular smooth muscle cell complex.

11. (Twice Amended) A method for [introducing a gene of interest into a mammalian subject] preparing a vascular prosthesis seeded *ex vivo* with vascular smooth muscle cells transduced to express a gene of interest, comprising the steps of:

[engrafting a device as in claim 1 into the subject's vascular system, wherein the transduced autologous] transducing mammalian vascular smooth muscle cells [contain] with the gene of interest operably linked to a promoter for expression;

and immobilizing the transduced vascular smooth muscle cells on a vascular graft surface, whereby the smooth muscle cells remain stably immobilized on the graft surface and express a product of said gene.

13. (Twice Amended) A method as in claim 11, wherein the [device is engrafted into the subject's arterial system] gene encodes erythropoietin.

14. (Twice Amended) A method [for delivering erythropoietin to a mammalian subject, comprising engrafting a device as in claim 1 into the subject's vascular system, wherein the transduced autologous smooth muscle cells express erythropoietin] as in claim 11, wherein the gene encodes Factor IX.

15. (Twice Amended) The method of claim [14, wherein the device is engrafted into the subject's arterial system] 11, wherein the gene encodes granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor.

16. (Twice Amended) A method [for treating an occlusion of a blood vessel in a mammalian subject, comprising engrafting a device as in claim 1 into the occluded blood vessel bypassing the occlusion] as in claim 11, wherein the transduced cells constitutively express an anticoagulant protein.

19. (Three Times Amended) A method [for delivering an] as in claim 11, wherein the gene of interest encodes glucose-regulated insulin or proinsulin polypeptide [to a mammalian subject, comprising engrafting a device as in claim 1 into the subject], and wherein the transduced cells [constitutively] express glucose-regulated [an] insulin or proinsulin polypeptide.

20. (Twice Amended) A method for [delivering] preparing a vascular prosthesis seeded *ex vivo* with vascular smooth muscle cells transduced to express a protein product [to a mammalian subject], comprising the steps of:

[removing] culturing vascular endothelial cells and vascular smooth muscle cells [from the subject];

transducing the smooth muscle cells with a gene which encodes the protein product, operably linked to a promoter;

immobilizing on a tubular elongate porous vascular graft device the transduced smooth muscle cells within the pores and interior surface of the graft; and

coating the interior of the graft device having immobilized thereon the transduced smooth muscle cells with the endothelial cells[; and

engrafting the device having the immobilized transduced smooth muscle cells and endothelial cells into the vasculature of the subject to deliver the protein product thereto].

21. (Twice Amended) The method of claim 20, further comprising the step of cultivating the vascular smooth muscle cells obtained from [the] a mammalian subject in a medium containing autologous serum prior to immobilizing the cells on the vascular graft.

22. (Amended) The method of claim 21, further comprising the step of cultivating the vascular endothelial cells obtained from a mammalian subject in a medium containing autologous serum prior to coating the vascular graft.